SEROLOGICAL SYSTEMATICS OF SOME PALINURAN AND ASTACURAN CRUSTACEA[1]

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Introduction

Only a few serological tests indicating the taxonomic relationships among the long-tailed Crustacea are recorded in the literature. Nuttall (1904) and Graham-Smith (1904), both using the same antisera made against *Homarus vulgaris* and *Potamobius astacus,* separately tested these antisera with a large variety of animal sera; of these antigens only those of the decapod Crustacea gave positive reactions. The results obtained by these authors, in some instances, were not in accord with accepted zoological classifications. The serum of *Palinurus vulgaris,* for example, reacted more weakly with the anti-*Homarus vulgaris* serum than did the sera of certain brachyuran species. Erhardt (1929) produced an antiserum against the serum of the crayfish *Potamobius astacus* and tested it with the homologous serum, plus the sera of *Potamobius leptodactylus,* the lobster *Homarus vulgaris,* several brachyuran species, and other invertebrates. Except for an unexplained heterologous reaction with the serum of the snail, *Helix pomatia,* only the crayfishes and lobster reacted, and these in accordance with the taxonomic positions previously assigned to them on the basis of information obtained from non-serological data. That the sera used as antigens by these early workers were not standardized in terms of protein content may, in part, explain the unexpected results sometimes obtained when the reactivity of their apparently non-comparable antigen dilutions were compared.

Clark and Burnet (1942) using the ring test sensitivity method, and unstandardized antigen dilution series, were able to separate various genera of the Parastacidae from each other and from other Decapods.

In 1943, Boyden, using a turbidimetric analysis of the whole precipitin range, measured the degree of serological correspondence between the sera of *Homarus americanus* and *Homarus vulgaris* with an appropriate antiserum. The index of serological activity used by this author was a value proportionate to the whole curve area. This was the first application of a quantitative technique to obtain serological data that was to be used in judging the taxonomic relationships of the long-tailed Crustacea.

The present writer made a preliminary report (Leone, In press) of his investigations of the sera of invertebrates, and included in it some of the results reported below. The object of this present report is to record further serological data that

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will aid in making a more accurate taxonomic arrangement of the palinuran and astacuran Crustacea.

Materials and Methods

As a result of the establishment of the Serological Museum of Rutgers University (Boyden, 1948), cooperating institutions and individuals from several parts of the world have aided in collecting and providing the samples of animal sera used in this investigation. Sera of species of the Australian genera *Euastacus* and *Cherax* were obtained through the efforts of Miss Ellen Clark of Victoria. Sera of the Hawaiian species of *Panulirus* were provided by Dr. Bradley T. Scheer of the University of Hawaii. Sera of the American west-coast spiny lobster were provided by Dr. Albert Tyler, California Institute of Technology, Pasadena, California. The other samples of sera came from stocks in the Serological Museum, which were collected chiefly by Dr. Alan A. Boyden, Rutgers University. Table I contains a list of all the antigens used and the antisera prepared.

Table I

A list of the species, and their sources, whose sera were used in making the serological comparisons reported upon in this paper

Species	Sample	Source	Antiserum
Panulirus argus (Latreille)	39–1 ♂	Tortugas, Florida, U.S.A.	I–76
Panulirus argus (Latreille)	39–3 ♂	Tortugas, Florida, U.S.A.	I–77
Panulirus interruptus (Randall)	46–1	California Institute of Technology, California, U.S.A.	I–56
Panulirus penicillatus (Oliver)	49–1	Honolulu, Hawaii	I–117
Panulirus japonicus (de Siebold)	49–1	Honolulu, Hawaii	
Palinurus vulgaris Latreille	No. 1	Plymouth, England	I–93
Homarus vulgaris Milne-Edwards	No. 1	Plymouth, England	I–91
Homarus americanus Milne-Edwards	36–2d	Mt. Desert Island, Maine, U.S.A.	I–89
Cambarus clarkii Girard	36–1	(Unknown)	I–92
Euastacus elongatus Clark	48–1	Victoria, Australia	I–116
Euastacus nobilis(Dana)	48–1	Victoria, Australia	
Euastacus armatus (von Martens)	48–1	Victoria, Australia	
Cherax destructor Clark	48–1	Victoria, Australia	
Cherax albidus Clark	48–1	Victoria, Australia	

The details of the technique of testing have been considered elsewhere (Boyden, 1942; Boyden and DeFalco, 1943; Leone, 1949). Briefly, the method employed is to measure, by means of a photoelectric turbidimeter, the precipitate developed in an antigen dilution series, after a constant amount of antiserum has been added to each dilution. The range of antigen concentrations extends from complete antigen excess to complete antiserum excess. Also considered in these earlier papers were various problems related to establishing the validity of the results of tests made with sera collected over a period of years, and which were processed in various ways. In general, the serum antigens proved to be representative of the organisms from which they came, and were suitable reagents with which to conduct researches in serological systematics. The customary twenty-minute reaction time at 38° C. was employed in these tests. In examining the degree of serological correspond-

ence [3] among the sera of the various species in the genera *Panulirus* and *Euastacus* a twenty-four hour reaction time at room temperature was employed, as well as the short time readings.

Experimental Results

The results of the serological tests are summarized in Table II and Table III.

Table II

Serological Relationships

Antigens	Antisera							
	Panulirus argus	*Panulirus interruptus*	*Panulirus penicillatus*	*Palinurus vulgaris*	*Homarus americanus*	*Homarus vulgaris*	*Cambarus clarkii*	*Euastacus elongatus*
Panulirus argus	100	86	88	35	5	5	6	4
Panulirus interruptus	85	100	88	31	5	5	5	4
Panulirus penicillatus	88	88	100	33	4	4	4	4
Panulirus japonicus	70	71	77		13		4	
Palinurus vulgaris	54	31	50	100	4	7	5	3
Homarus americanus	14	9	15	5	100	96	50	10
Homarus vulgaris	15	11	16	6	88	100	48	10
Cambarus clarkii	13	11	14	6	50	48	100	38
Euastacus elongatus	13	11	14	6	54	49	57	100
Euastacus nobilis	13	10	14	7	53	49	58	89
Euastacus armatus	13	11	13	7	53	47	55	88
Cherax destructor	12	9	11	6	47	47	65	61
Cherax albidus	10	8	12	6	48	45	63	58

The numbers in the table are percentages of the value of the homologous reaction which is designated as 100 per cent. Values within 5 per cent of one another are generally considered to be within the experimental error of the method. All values are averaged from two or more tests, except those for *Panulirus japonicus*. It is readily seen that the various antisera differ in their capacity to react with the heterologous sera. All values are for 20 minute reaction time.

Of the species tested in the genus *Panulirus,* a high degree of correspondence is shown by the sera of the spiny lobsters, *P. argus, P. interruptus,* and *P. penicillatus*. On the basis of the serological evidence alone, these three species are in the same category, or are in closely related categories, whereas *P. japonicum* is in another category.[4] The European spiny lobster, *Palinurus vulgaris,* is in still a different category and the degree of difference between it and the others is so great as to suggest differences on the generic level.

[3] Serological correspondence. As applied to antigens it includes all antigens capable of reacting with an antiserum to any one of them. As applied to antibodies, or antisera, it includes all antibodies or antisera capable of reacting with a given antigen or hapten. Whenever the serological correspondence is exact we may speak of serological equivalence (Boyden, 1942).

[4] The serum sample of this species had a soluble protein concentration of less than one per cent, and, in addition, was preserved in merthiolate colored with eosin. Consequently the contents of the tubes in the antigen excess region of the reaction curve were dark red. The amount of reaction recorded seemed to be less than a visual estimate of the dilution tubes would indicate. The antiserum excess region of the curve was normal. Any change, applied as a correction, would be in the direction of a closer correspondence to the homologous antigen indicating less difference between *P. japonicum* and the other palinuran species than is indicated in the table.

TABLE III

A comparison of 20 minute and 24 hour reaction times

Species	Reaction time					
	20′	24 hr.	20′	24 hr.	20′	24 hr.
Panulirus penicillatus	100	100	88	100	86	96
Panulirus argus	88	102	100	100	85	99
Panulirus interruptus	87	100	88	99	100	100
Euastacus elongatus	100	100				
Euastacus armatus	88	98				
Euastacus nobilis	89	96				

Values indicate the per cent relationship to the homologous reaction. The antigenic correspondence indicated after 20 minutes is high; after 24 hours correspondence in antigen reaction is exact.

The true lobsters, *Homarus americanus* and *Homarus vulgaris,* also show a high degree of serological correspondence. Whether or not the difference between them is sufficient to merit separate specific status for each is open to question from the serological point of view.

The three species of the genus *Euastacus* closely correspond to one another serologically, the heterologous reactions being only 11 to 12 per cent less than the homologous reaction. As it was in the cases of the palinuran organisms, these astacuran species show less than the 30–50 per cent difference from the homologous reaction which has been suggested (Boyden, 1943) as defining species in serological tests involving decapod Crustacea. As Boyden pointed out (personal communication), these values depend upon the grade of specificity of the antisera used and have reference to comparable antisera only.

The serum of *Cambarus clarkii* is notably different from the sera of the other astacuran species. The sera of species of the fresh water genera *Euastacus* and *Cherax* react more strongly with the anti-*Cambarus* serum than do the sera of the true lobsters of the genus *Homarus.* The sera of *Homarus,* on the other hand, react considerably more with anti-*Cambarus* serum than do the sera of any of the palinuran, spiny lobsters. Sera of species of the genus *Cherax* gave slightly greater reactions with the anti-*Euastacus* serum than did the serum of *Cambarus.*

Various species of brachyuran Crustacea were tested with each of the antisera shown in Table I. Heterologous reactions were obtained in some tests, indicating serological correspondence. In no test, however, did the serum reaction of any brachyuran exceed, or approach for that matter, the weakest heterologous reaction of the palinuran and astacuran species.

In order to ascertain whether the differences in the reactivity of heterologous antigens of high serological correspondence were constant, some of the values for twenty-minute reaction times were compared with twenty-four hour reaction times. A period of twenty-four hours at room temperatures was sufficient time for the precipitin reactions to go to completion. Merthiolate employed at a concentration of 1 : 10,000 in the saline diluent was adequate to inhibit bacterial growth during the test period.

From the data in Table III it can be seen that the heterologous test values which differ from the homologous by as much as fifteen per cent after twenty-minutes reaction time, may appear to be serologically equivalent after twenty-four hours of reaction time. Reactions with values within five per cent of the homologous value are considered equivalent. The sera of the three listed species of the genus *Panulirus* appear to be serologically equivalent, after twenty-four hours, as are the sera of the three species of the genus *Euastacus*. When the correspondence of the sera of heterologous species is as high as it was in these tests, experience has taught us that detectable differences, as revealed by other criteria, are slight. Consequently the usefulness of serum as a precise taxonomic tool is demonstrated. These observations are pertinent for the sera of Crustacea which possess only a single kind of antigenic protein, the hemocyanins (Allison and Cole, 1940; Clark and Burnet, 1942; Tyler and Scheer, 1945), and are, by their nature, relatively pure serological reagents. Serological reactions with these sera are not so subject to the inconsistencies and irregularities which sometimes are revealed, with proper techniques, in mixed antigens such as the sera of mammals which contain several antigenically distinct proteins (Serological Museum, Bulletin No. 2, 1948).

Summary

From the serological studies of the palinuran and astacuran Crustacea it is concluded that:

1. Sera of species within a genus react more strongly with an antiserum made against one of them than with any other antiserum.
2. Sera of different species within the same genus may show different degrees of relationship to an antiserum made against the serum of one member of that genus.
3. The sera of representatives of different genera in the same family show different degrees of relationship to one another.
4. The sera of species within a family react to a greater extent with antisera made against the sera of members of that family than with any other antisera.
5. *Panulirus argus, Panulirus interruptus,* and *Panulirus penicillatus* correspond almost exactly with one another serologically, and on the basis of the serological evidence presented, may be considered the same, or closely related species. This is in spite of their wide separation geographically.
6. *Euastacus elongatus, Euastacus nobilis,* and *Euastacus armatus,* serologically, closely correspond with one another. Probably they are the same, or closely related, species.
7. In the genus *Homarus,* the two species *H. americanus* and *H. vulgaris* closely correspond with each other, and probably are the same, or closely related species.
8. Differences in per cent of serological relationship among crustacean sera tested with the same antiserum are attributable to differences in their hemocyanins.
9. Twenty-minute reaction times for precipitin reactions establish significant orders of relationship among animal sera. Twenty-four hours reaction time does not alter the order of relationship from that established at twenty minutes despite considerable increases in amounts of reaction recorded.

10. Differences detectable among antigens of high correspondence, after a twenty-minute reaction time, may disappear after a reaction time of twenty-four hours.

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